Supporting Information

for

Controlling the Shape of Filamentous Cells of *Escherichia Coli*

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Materials. Tryptone was purchased from Difco; agarose was purchased from Invitrogen (Ultrapure). The *N*-hydroxysuccinimide ester of Cy 3 was purchased from Amersham Biosciences. All other chemicals were purchased from Sigma unless otherwise noted, and used as received. MilliQ water and *E. coli* strain AW405[1] were used in these studies.

Growth of bacterial cultures. Saturated cultures of *E. coli* were grown in TB broth (1% tryptone, 0.5% sodium chloride, pH 7.0) for 14-16 hr at 31-33 °C on a rotary shaker (150 rpm); aliquots were frozen in 15% glycerol at -78 °C and stored at the same temperature. To grow motile suspensions used for these studies, saturated cultures were thawed and diluted 1:100 into 5 mL of fresh TB broth. Cells were then grown at 31-33 °C on a rotary shaker (150 rpm) for 2-4 hr.

Preparation of agarose microchambers. Silicon masters containing features in photoresist (SU8, MicroChem Corp.) in bas relief were fabricated by photolithography. Agarose substrates with embossed features were prepared by casting hot, sterile media (~65 °C) containing 2% (w/v) agarose onto silicon masters or poly(dimethylsiloxane) (PDMS) replicas of silicon masters.[2] Media contained 1% tryptone, 0.5% sodium chloride, 0.05% BSA, and 20 μg/mL cephalexin. We allowed the agarose to gel at room temperature for at least 15 minutes before we peeled the agarose layer away from the silicon master or PDMS replica. A suspension of *E. coli* (5 μL, ~ 10^4 cells/μL) was added to the patterned agarose stamp. A thin, PDMS film (150 μm thick) or an agarose slab (1% tryptone, 0.5% sodium chloride,

0.05% BSA, 20 μL/mL cephalexin, and 0.5% w/v agarose) was placed gently on top of the chamber to confine cells.

Image Acquisition and Data Analysis. Agarose chambers and cells were observed using phase-contrast microscopy using a Nikon E400 or a Leica DMIRB microscope with a heated microscope stage (Research Instruments Limited). Still images were collected with a digital camera (Hamamatsu Orca-ER) using Metamorph software. Video images of translating cells were acquired using a black and white CCD camera (Marshall Electronics V1070) connected to a digital video recorder (Sony GV-D1000) that collected at images at 30 frames per second. Video was captured using Adobe Premiere and analyzed using Scion Image (available for download at www.scioncorp.com) or ImageJ (available for download at http://rsbweb.nih.gov/ij/). Still images collected from videos were processed by subtracting out the background and adjusting the brightness and contrast. The speed of cells was measured by taking a six-second average of the distance that the center-point of the cell traveled between each frame. Fluorescence microscopy was performed using a Leica DMIRB microscope, a mercury lamp, and Rhodamine filter cube. Fluorescent images were obtained with a monochrome CCD camera (Marshall Electronics).

Fluorescent Labeling Procedure. Shaped filamentous cells were labeled according to the procedure by Turner *et al.*[3] Cells were washed from growth media into motility buffer (10 mM potassium phosphate, pH 8.1, 20 μg/mL cephalexin) by centrifugation (500g) three

times. Cy3 monofunctional reactive dye (50 μg, Amersham) was dissolved in motility buffer (100 μL) and added to a cell pellet that had been resuspended in 100 μL of motility buffer. Sodium bicarbonate (10 μL, 0.1 M) was added to bring the pH to ~8.3. Cells were gently shaken in the solution of dye for 1 hr, and then were separated from the dye by centrifugation three times (500g).

(1) Armstrong, J. B.; Adler, J.; Dahl, M. M. *J. Bacteriol.* **1967**, *93*, 390-398.
(2) Xia, Y.; Whitesides, G. M. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 550-575.
(3) Turner, L.; Ryu, W. S.; Berg, H. C. *J. Bacteriol.* **2000**, *182*, 2793-2801.